



## Carbon and nitrogen elemental and isotopic ratios of filter-feeding bivalves along the French coasts: An assessment of specific, geographic, seasonal and multi-decadal variations



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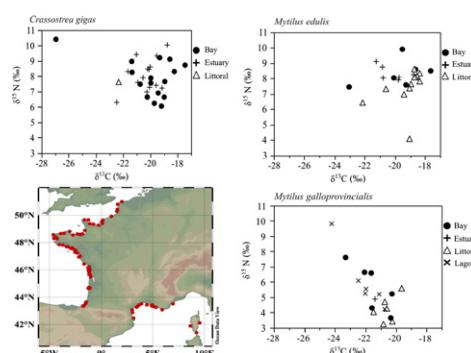
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### HIGHLIGHTS

- Trophic status of coastal ecosystems explains the geographical variation of bivalve  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .
- Seasonal variability in C/N is related to reproduction cycle for mussel species.
- $\delta^{13}\text{C}$  seasonal variability is related to trophic resource variability for *C. gigas* and *M. edulis*.
- Multi-decadal shifts and trends in C/N ratio,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were detected and likely related to climate change.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Primary consumers play a key role in coastal ecosystems by transferring organic matter from primary producers to predators. Among them, suspension-feeders, like bivalve molluscs are widely used in trophic web studies. The main goal of this study was to investigate variations of C and N elemental and isotopic ratios in common bivalves (*M. edulis*, *M. galloprovincialis*, and *C. gigas*) at large spatial (i.e. among three coastal regions) and different temporal (i.e. from seasonal to multi-decadal) scales in France, in order to identify potential general or specific patterns and speculate on their drivers.

The observed spatial variability was related to the trophic status of the coastal regions (oligotrophic Mediterranean Sea versus meso- to eutrophic English Channel and Atlantic ocean), but not to ecosystem typology (estuaries, versus lagoons versus bays versus littoral systems). Furthermore, it highlighted local specificities in terms of the origin of the POM assimilated by bivalves (e.g., mainly continental POM vs. marine phytoplankton vs. microphytobenthic algae). Likewise, seasonal variability was related both to the reproduction cycle for C/N ratios of *Mytilus* spp. and to changes in trophic resources for  $\delta^{13}\text{C}$  of species located close to river mouth. Multi-decadal evolution exhibited shifts and trends for part of the 30-year series with decreases in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Specifically, shifts appeared in the early 2000's, likely linking bivalve isotopic ratios to a cascade of processes affected by local drivers.

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## 1. Introduction

Representing significant aquaculture resources on a worldwide level, bivalve molluscs such as mussels or oysters are species of high economic interest (FAO – Fisheries and Aquaculture Department). As filter-feeding animals and primary consumers, they also constitute a major component of coastal trophic network and ecosystem functioning. However, being mainly located in coastal areas, they are subject to a highly variable environment (e.g. water temperature, salinity, river discharges, inputs of chemical contaminants etc.). These variable environmental conditions can notably impact the quality (origin, composition) of their food sources and their physiological state (Hawkins et al., 1985; Thompson and Harrison, 1992; Prins et al., 1997; Gasmi et al., 2017).

As filter-feeders, bivalves rely on biological material suspended in the water column including detritus, (micro)algae, and other microorganisms. Several studies showed that bivalves from estuarine and coastal environments are subject to a fluctuating and unpredictable food supply (e.g. Kreeger et al., 1988; Crosby et al., 1990), especially depend on the season. In addition to marine phytoplankton, suspended particulate organic matter (POM) originating from river discharge can thus represent a significant food source for estuarine and coastal bivalves (Hackney and Haines, 1980; Stephenson and Lyon, 1982; Riera and Richard, 1996a). River-derived POM includes a mix of freshwater phytoplankton, sloughed-off periphyton, terrestrial POM (i.e. vascular plant detritus, soil) and anthropogenic (i.e. waste-water) POM (Raikow and Hamilton, 2001; Savoye et al., 2012; Liénart et al., 2016). However, phytoplankton is generally considered as a high-quality food for bivalves, and variability in this resource can impact the ecological performance of these primary consumers in the field (e.g. size, weight...) (Kiorboe and Mohlenberg, 1981; Blanton et al., 1987; Grant, 1996; Cranford and Hill, 1999).

There are inherent difficulties to accurately assess the food sources ingested and/or assimilated by filter-feeding or planktivorous organisms using traditional methods, such as of stomach or gut contents analyses. In the last decades, the analysis of carbon (C) and nitrogen (N) stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values) in biological tissues has proven to be a useful approach for identifying the relative contribution of the different food sources in the diet of estuarine and coastal primary consumers (e.g., Simenstad and Wissmar, 1985; Peterson and Fry, 1987; Fry and Sherr, 1984; Currin et al., 1995; Lefebvre et al., 2009; Dubois et al., 2014). Indeed, the different primary producers and/or POM components (e.g. material from terrestrial vs. marine origin, fresh vs. detrital material, etc.) generally display different isotopic compositions (e.g., Dubois et al., 2014). Furthermore, C and N stable isotope compositions of consumers' tissues have been shown to closely resemble those of their food sources (De Niro and Epstein, 1978; De Niro and Epstein, 1981). Also, the enrichment between sources and consumers is relatively predictable (De Niro and Epstein, 1978; Minagawa and Wada, 1984; Peterson and Fry, 1987), although it may vary depending on the tissues considered, taxa, food sources, physiological state of consumers, etc. (e.g. Lorrain et al., 2002; Emmery and Lefebvre, 2011). Nonetheless, across such bivalve species as mussels and oysters, this enrichment (usually called "trophic enrichment factor", TEF) has been reported to be quite consistent (Dubois et al., 2007a,b). Moreover, the bulk C/N ratio measured in biological tissues (as well as  $\delta^{13}\text{C}$  values to a lesser extent) may inform on the lipid content and/or the physiological state (i.e., repletion status) of organisms. Lipids are effectively enriched in carbon chains and depleted in  $^{13}\text{C}$  relative to other tissue components (De Niro and Epstein, 1978; Bodin et al., 2007). Since bivalves are primary consumers and constitute a major component of littoral trophic networks and ecosystems, their stable isotope ratios are used as "baseline" isotopic values for local biological communities (e.g. Abrantes and Sheaves, 2009). Indeed bivalves may represent useful model organisms able to exhibit time-integrated isotopic values relative to local primary producers, or local POM composition and origin.

Carbon and nitrogen elemental and isotope compositions of various bivalve species in France have already been described and extensively studied for some specific and local spots (e.g. Riera and Richard, 1996a,b; Lefebvre et al., 2009; Dubois et al., 2014). It is the first study to examine C and N elemental and isotope compositions at large spatial and temporal scales, probably due to difficulties in adequate sample acquisition and curation. In this general context, the present study proposes to achieve for the first time an instantaneous global cartography of C/N ratios,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values measured in bivalves collected in 2014 along the coastline belonging to three regions and to a large diversity of ecosystems and environmental conditions, and for selected sites, over three decades. The specific objectives of this study were to investigate (i) spatial (ii) seasonal and (iii) multi-decadal variations in C and N elemental and isotopic ratios of bivalves in order to identify potential general trends and environmental drivers of bivalve food resources and physiological status. As companion paper of a two mercury papers (Briant et al., 2017),  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were used as proxies of food sources and the C/N ratio as a proxy of body condition. The study was performed along the three regions of the French coastal ocean between 1987 and 2014.

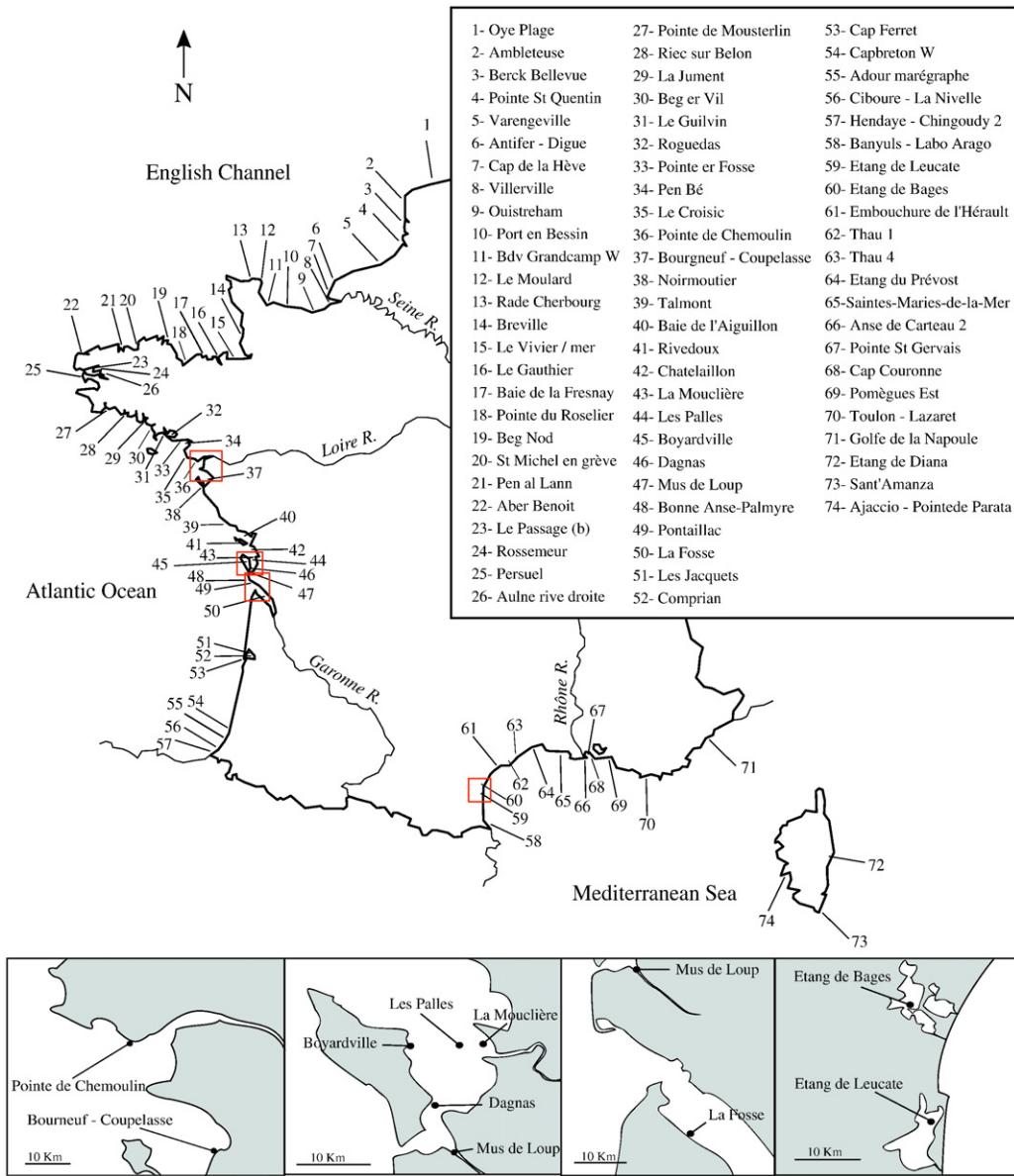
## 2. Materials and methods

### 2.1. Study sites and sampling strategy

Since the end of 1970's, a French Mussel-Watch program, the National Monitoring Network "ROCCH" (for "Réseau d'Observation de la Contamination Chimique") uses wild local bivalves as biomonitor and semi-quantitative indicators of the chemical contamination of the national coastline (Claisse, 1989). Currently, the network includes nearly 80 stations distributed along the coastline of continental France and the French Antilles. At each station, similar-sized bivalves are collected twice a year (in autumn and in winter). In the first years and until the early 2000's, the stations were sampled all four seasons.

The analyses presented in this study concern 189 samples of bivalves from 74 sampling stations chosen along the three maritime façades of metropolitan France (the English Channel, the Atlantic Ocean, the Mediterranean Sea) (Fig. 1). Sites have been selected to sample the high diversity of environmental conditions encountered by the bivalves along the coastline: sites are located in estuaries, semi-enclosed systems, open bays, lagoons, rocky or sandy-bottom areas, in eutrophic, mesotrophic or oligotrophic systems, are subject to temperate oceanic or Mediterranean climate, etc. (Table 1). In this study, following criteria of the ROCCH program, four locations were distinguished. "Littoral" characterizes each open sea site that can be rock or sand ground; "estuary" is used when the site is at the vicinity of a coastal river mouth, whatever the size of the river; "bay" represents each site within a semi-enclosed area; and "lagoon" is typical to some Mediterranean sites (almost closed lagoons). The four cases allow to describe the general type of environment and associated hydrological dynamic for the different sites. Despite the contrasts between these environments, only three different species of bivalves were collected: the mussel *Mytilus edulis* in the English Channel and the Atlantic Ocean, the mussel *Mytilus galloprovincialis* in the English Channel and the Mediterranean Sea, and the oyster *Crassostrea gigas* in the English Channel and the Atlantic Ocean (Fig. 1).

The seasonal variability was investigated using results from *M. edulis*, *C. gigas*, and *M. galloprovincialis* sampled during winter, spring, summer and fall of 1998 and 1999 in the three sites: Pointe Chemoulin and Bourgneuf-Couplasse in the Atlantic Ocean, and Cap Couronne in the Mediterranean Sea. The spatial variability was investigated using samples of the three species sampled at the 74 sites during winter (between late January and early March) 2014. Finally, the decadal variability was investigated using samples of the three species sampled each three years from winter 1987 to winter 2014 in ten sites of the three



**Fig. 1.** Geographic location and names of the sampling sites. Insets (left to right) detail red square sites from north to south.

facades. The ten sites were selected on the three facades with respect to the ecosystem and species diversities.

## 2.2. Sample collection and treatment

The identical sampling protocol has consistently been applied to bivalves collected since 1979 within the framework of ROCCH programme (formerly known as RNO). Briefly, each mussel sample was constituted of a pool of fifty individuals (35–65 mm in shell length representing 2–3 years of growth), while oyster samples represented 10 cultivated individuals of two to three years old. After collection, bivalves were cleaned of epibionta and depurated for 24 h in a polyethylene aquarium containing decanted water from the sampling site, in order to eliminate faeces, pseudofaeces and potential inorganic material ingested from their digestive tracts. Then, their soft tissues were removed from shells, placed in a combusted glass jar, frozen (−20 °C) within 24 h, and sent to the central laboratory in Nantes within a week. There, samples were thawed, homogenized with a stainless steel-bladed blender, frozen again (−20 °C), and finally freeze-dried

in acid-clean glass jars. The protocol is fully described in [Claisse \(1989\)](#). Samples (dried and homogenized powders) were stored in a dark and moisture-regulated room until chemical analyses.

## 2.3. Elemental and isotopic analyses

Aliquots of 400 µg of each sample were analysed at the EPOC Laboratory (Talence/Bordeaux, France) using an Elemental Analyzer (NC2500, CarloErba®) connected to an Isotope Ratio Mass Spectrometer (Isoprime, GV Instruments®). Isotopic data are expressed using the usual  $\delta$  notation ([Coplen, 2011](#)) with a per mil deviation (‰) from international reference materials (Vienna Pee Dee belemnite for  $\delta^{13}\text{C}$  and atmospheric  $\text{N}_2$  for  $\delta^{15}\text{N}$ ). Data were corrected and calibrated against homemade working standards (casein) and certified standards (acetanilide, IAEA-N2, USGS-24). These standards allowed our analytical precision to be set at  $\leq 0.2\%$  for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and  $\leq 0.2 \text{ mol mol}^{-1}$  for C/N ratio. It should be noted that C/N ratio is expressed in  $\text{mol mol}^{-1}$  throughout the manuscript.

**Table 1**  
Site specificities.

Number	Name	Species	Ecosystem	Ocean/sea
1	Oye plage	<i>Mytilus edulis</i>	Littoral	English Channel
2	Ambleteuse	<i>Mytilus edulis</i>	Littoral	English Channel
3	Berck Bellevue	<i>Mytilus edulis</i>	Littoral	English Channel
4	Pointe de St Quentin	<i>Mytilus edulis</i>	Littoral	English Channel
5	Varengeville	<i>Mytilus edulis</i>	Littoral	English Channel
6	Antifer - digue	<i>Mytilus edulis</i>	Littoral	English Channel
7	Cap de la Hève	<i>Mytilus edulis</i>	Estuary	English Channel
8	Villerville	<i>Mytilus edulis</i>	Estuary	English Channel
9	Ouistreham	<i>Mytilus edulis</i>	Estuary	English Channel
10	Port en Bessin	<i>Mytilus edulis</i>	Littoral	English Channel
11	Bdv Grandcamp ouest	<i>Mytilus edulis</i>	Bay	English Channel
12	Le Moulard	<i>Mytilus edulis</i>	Littoral	English Channel
13	Grande rade de Cherbourg	<i>Mytilus edulis</i>	Bay	English Channel
14	Bréville	<i>Mytilus edulis</i>	Littoral	English Channel
15	Le Vivier sur mer	<i>Mytilus edulis</i>	Bay	English Channel
17	Baie de la Fresnaye	<i>Mytilus edulis</i>	Bay	English Channel
20	St Michel en grève	<i>Mytilus edulis</i>	Bay	English Channel
27	Pointe de Mousterlin	<i>Mytilus edulis</i>	Littoral	Atlantic ocean
29	La Jument	<i>Mytilus edulis</i>	Estuary	Atlantic ocean
34	Pen Bé	<i>Mytilus edulis</i>	Bay	Atlantic ocean
35	Le Croisic	<i>Mytilus edulis</i>	Littoral	Atlantic ocean
36	Pointe de Chemoulin	<i>Mytilus edulis</i>	Estuary	Atlantic ocean
43	La Mouclière	<i>Mytilus edulis</i>	Estuary	Atlantic ocean
54	Capbreton ouest	<i>Mytilus edulis</i>	Littoral	Atlantic ocean
19	Beg Nod	<i>Crassostrea gigas</i>	Littoral	English Channel
21	Pen al Lann	<i>Crassostrea gigas</i>	Bay	English Channel
22	Aber Benoît	<i>Crassostrea gigas</i>	Estuary	English Channel
23	Le Passage (b)	<i>Crassostrea gigas</i>	Estuary	Atlantic ocean
24	Rossermeur	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
25	Persuel	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
26	Aulne rive droite	<i>Crassostrea gigas</i>	Estuary	Atlantic ocean
28	Riec sur Belon	<i>Crassostrea gigas</i>	Estuary	Atlantic ocean
30	Beg er Vil	<i>Crassostrea gigas</i>	Estuary	Atlantic ocean
31	Le Guilvin	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
32	Roguedas	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
33	Pointe er Fosse	<i>Crassostrea gigas</i>	Estuary	Atlantic ocean
37	Bourgneuf - Coupelasse	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
38	Noirmoutier - Gresse-loup	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
39	Talmont	<i>Crassostrea gigas</i>	Estuary	Atlantic ocean
40	Baie de l'Aiguillon	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
41	Rivedoux	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
42	Châtelailleur	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
44	Les Palles	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
45	Boyardville	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
46	Dagnas	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
47	Mus de loup	<i>Crassostrea gigas</i>	Estuary	Atlantic ocean
48	Bonne Anse - Palmyre	<i>Crassostrea gigas</i>	Estuary	Atlantic ocean
49	Pontaillac	<i>Crassostrea gigas</i>	Estuary	Atlantic ocean
50	La Fosse	<i>Crassostrea gigas</i>	Estuary	Atlantic ocean
51	Les Jacques	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
52	Comprian	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
53	Cap Ferret	<i>Crassostrea gigas</i>	Bay	Atlantic ocean
55	Adour marégraphe	<i>Crassostrea gigas</i>	Estuary	Atlantic ocean
56	Ciboure - la Nivelle	<i>Crassostrea gigas</i>	Estuary	Atlantic ocean
57	Hendaye - Chingoudy 2	<i>Crassostrea gigas</i>	Estuary	Atlantic ocean
16	La Gauthier	<i>Mytilus galloprovincialis</i>	Estuary	English Channel
18	Pointe du Roselier	<i>Mytilus galloprovincialis</i>	Bay	English Channel
58	Banyuls - Labo Arago	<i>Mytilus galloprovincialis</i>	Littoral	Mediterranean sea
59	Etang de Leucate	<i>Mytilus galloprovincialis</i>	Lagoon	Mediterranean sea
60	Etang de Bages	<i>Mytilus galloprovincialis</i>	Lagoon	Mediterranean sea
61	Embouchure de l'Hérault	<i>Mytilus galloprovincialis</i>	Littoral	Mediterranean sea
62	Thau 1	<i>Mytilus galloprovincialis</i>	Lagoon	Mediterranean sea
63	Thau 4	<i>Mytilus galloprovincialis</i>	Lagoon	Mediterranean sea
64	Etang du Prévost	<i>Mytilus galloprovincialis</i>	Lagoon	Mediterranean sea
65	Les Stes Maries de la mer	<i>Mytilus galloprovincialis</i>	Littoral	Mediterranean sea
66	Pointe St Gervais	<i>Mytilus galloprovincialis</i>	Bay	Mediterranean sea
67	Anse de Carteau 2	<i>Mytilus galloprovincialis</i>	Bay	Mediterranean sea
68	Cap Couronne	<i>Mytilus galloprovincialis</i>	Littoral	Mediterranean sea
69	Pomègues Est	<i>Mytilus galloprovincialis</i>	Littoral	Mediterranean sea
70	Toulon - Lazaret	<i>Mytilus galloprovincialis</i>	Bay	Mediterranean sea
71	Golfe de la Napoule	<i>Mytilus galloprovincialis</i>	Bay	Mediterranean sea
72	Etang de Diana	<i>Mytilus galloprovincialis</i>	Lagoon	Mediterranean sea
73	Sant'Amanza	<i>Mytilus galloprovincialis</i>	Bay	Mediterranean sea
74	Ajaccio - Pte de Parata	<i>Mytilus galloprovincialis</i>	Littoral	Mediterranean sea

## 2.4. Data bases

In order to investigate the relationship between the core parameters ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and C/N ratio of bivalves) and environmental parameters considered as proxies of drivers, river flows were retrieved from BanqueHydro (<http://www.hydro.eaufrance.fr/>), and temperature, salinity and chlorophyll *a* concentration were retrieved from SOMLIT (<http://smlit.epoc.u-bordeaux1.fr/fr/>) and from QUADRIGE (<http://www.ifremer.fr/surval2/>) data bases. However, it should be noted that the latter set of parameters was not available for all studied sites and/or for the full studied long-term period, precluding detailed investigation of the relationship between the core and environmental parameters.

## 2.5. Statistical analyses

Geographical variability – Non-parametric Kruskal-Wallis tests followed by pairwise Wilcoxon post-hoc tests were used for comparing  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and C/N ratio between species at the countrywide scale and within coastal region. They were also used between coastal regions possessing all species and for each species, only using one-way test. Tests were not performed when any data set (size of a group within a single comparison) contained  $<3$  data points. To assess the spatial variability and search for any relationship between ecosystem type and isotope ratios, a permutational analysis of variance (PERMOANOVA; scaled data; Euclidean distances; one way unbalanced design) was performed on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  data for each species independently (Anderson, 2001). Multivariate homogeneity of group dispersion was previously verified.

Seasonal variability – Seasonal trends were tested with the hypothesis of a sinusoidal pattern. Sinusoidal models were fitted to the data using a least-squares regression. The trend was considered as following sinusoidal variation when the model was significant ( $p\text{-value} < 0.05$ ).

Multi-decadal variability – Monotonic trends were tested by Mann-Kendall corrected for auto-correlation (Yue and Wang, 2004) to relate each parameter ( $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and C/N ratio) to time. When significant, linear trends were tested and estimated by linear regression. Shifts were detected using Pettitt non-parametric test (Pettitt, 1979). All statistical

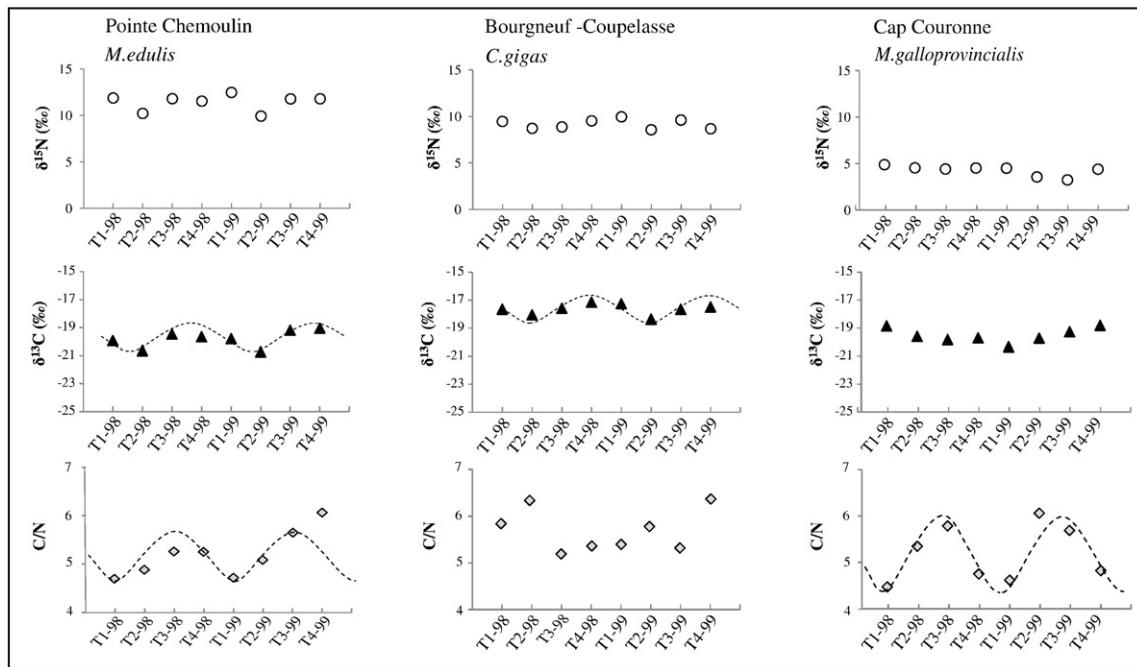
tests were performed using the R software (R Development Core Team, 2016) using the base and fume packages, except shift detections that were performed using the package XLStat 2015.

## 3. Results and discussion

### 3.1. Temporal variability, part I (seasonal variability)

Seasonal variation of C and N elemental and isotopic ratios is presented Fig. 2, identifying each season by their collection trimester for each of the three sites and associated species investigated. For the two consecutive years studied in this manner (1998–1999), ranges of C/N values,  $\delta^{13}\text{C}$  values and  $\delta^{15}\text{N}$  are summarized for the three species in the Table 2. Our results are therefore in good agreement with literature for  $\delta^{13}\text{C}$  values (Dubois et al., 2007a,b; Deudero et al., 2009; Riera and Richard, 1997; Riera et al., 2002a,b; Riera, 2007). The  $\delta^{15}\text{N}$  values of this study are in the upper range of literature values which varying between 4.3 and 9.4% for *M. edulis* (Dubois et al., 2007a,b; Riera, 2007) and 4.7 and 10.0% for *C. gigas* (Dubois et al., 2007a,b; Riera, 2007).

The seasonality of the elemental ratio C/N appeared to follow a sinusoidal cycle for *M. edulis* and *M. galloprovincialis* ( $R^2 = 0.67$ ,  $p\text{-value} = 0.01$  and  $R^2 = 0.91$ ,  $p\text{-value} < 0.01$  respectively), with high values in summer and low values in winter, but not for *C. gigas*. Elemental ratio C/N of *C. gigas* also varied during the annual cycle with a decrease in C/N ratio from T2 to T3, although it did not significantly follow a sinusoidal cycle. It is coherent with the variations of the dry flesh mass (DFM) observed and simulated by Thomas et al. (2016), where the maximum of the DFM often occurs between June and July. Notably, *C. gigas*, and *M. edulis*, which exhibit differing seasonal variabilities, were sampled  $<30$  km apart, both near the mouth of the Loire River. Furthermore, *M. galloprovincialis*, whose C/N ratio exhibits a sinusoidal cycle, was sampled in the Mediterranean Sea away from any river discharge. In contrast, this sinusoidal periodicity seems common to the two *Mytilus* species studied. It appeared to be phased with the already observed “condition index” cycle of bivalves (Lucas and Beninger, 1985; Okumuş and Stirling, 1998; Orban et al., 2002) and its reproduction cycle. The C/N ratio varies with the (storage) lipid content: since most lipids are



**Fig. 2.** Seasonal variations for Pointe Chemoulin site (*M. edulis*), Bourgneuf site (*C. gigas*) and Cap Couronne site (*M. galloprovincialis*). Dotted line is the significant theoretical sinusoidal model.

**Table 2**Comparison of ranges of C/N ratio,  $\delta^{13}\text{C}$  values and  $\delta^{15}\text{N}$  values for the three bivalves.

	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C/N (mol mol <sup>-1</sup> )	Ref
<i>M. edulis</i>	–20.7 to –19.0	9.9 to 12.5	4.7 to 6.0	This study
	–22.0 to –18.4	4.3 to 9.4		Dubois et al., 2007a,b; Riera, 2007
<i>C. gigas</i>	–18.4 to –17.1	8.6 to 10.0	5.2 to 6.4	This study
		4.7 to 10.0		Dubois et al., 2007a,b; Riera, 2007
<i>M. galloprovincialis</i>	–23.0 to –17.3			Riera and Richard, 1997; Riera et al., 2002a,b; Riera, 2007
	–20.3 to –18.1	3.2 to 4.9	4.5 to 6.0	This study
	–22.0 to –19.7			Deudero et al., 2009

composed of C but not of N, the C/N ratio increases when the amount of lipid increases. The reproduction cycle of bivalves governs the mass of stored lipids and various French and European documents such as VELYGER report or OSPAR convention effectively reported a minimum C/N for bivalves collected in winter.

The seasonal variation of  $\delta^{13}\text{C}$  also followed a sinusoidal-like cycle for the two species sampled near the mouth of the Loire River on the Atlantic coast (*M. edulis* at Pointe Chemoulin ( $R^2 = 0.66$ ;  $p$ -value = 0.01) and *C. gigas* at Bourgneuf-Couplasse ( $R^2 = 0.68$ ;  $p$ -value = 0.01)), with low values in spring and high values in autumn. However, the sinusoidal pattern did not fit for *M. galloprovincialis* sampled in the Mediterranean Sea, away from any river mouth. Variation in  $\delta^{13}\text{C}$  values of consumers can be due to either a variation in lipid content with lower values when the lipid content is higher (Bodin et al., 2007), or a shift in diet resources with different  $\delta^{13}\text{C}$  (Lefebvre et al., 2009). Since the seasonal variation of C/N ratio (used here as a proxy of lipid content) and  $\delta^{13}\text{C}$  were different according to sampling site and species, the lipid content alone cannot explain the temporal variability of  $\delta^{13}\text{C}$ . Thus, this should be most likely related to seasonal variations in the composition of food resources. In the vicinity of river plumes characterized with low  $\delta^{13}\text{C}$  of riverine particulate organic matter (POM), near-shore ecosystems also exhibit low  $\delta^{13}\text{C}$  of their winter coastal POM, probably due to the increased influence of river discharge, and the reverse pattern is observed during low river flow (Liénart et al., 2017). Thus, the seasonal cycle of *M. edulis* and *C. gigas*  $\delta^{13}\text{C}$  sampled in the vicinity of the Loire River mouth records the seasonal variation in diet resources with a higher contribution of river POM (river phytoplankton and/or terrestrial POM) in winter compared to summer. Such variations have been observed for other species of bivalves in estuaries (e.g. Page and Lastra, 2003) and bays (Lorrain et al., 2002). However, this sinusoidal variability was not observed for *M. galloprovincialis* sampled in the Mediterranean Sea, away from any river mouth. In contrast for  $\delta^{15}\text{N}$ , no significant pattern is observed, regardless of site and species.

Overall, the seasonal variations observed in the three selected sites require using the same sampling season to investigate spatial, year-to-year and decadal variability of bivalve elemental and isotopic values.

### 3.2. Spatial variability

All sites and all species considered, C/N ratios varied between 4.3 and 8.2,  $\delta^{13}\text{C}$  values between –27.0 and –17.5‰ and  $\delta^{15}\text{N}$  values between 3.3 and 10.4‰. The highest C/N ratio was measured in *C. gigas* from Le Passage, and the lowest for *M. galloprovincialis* at the Embouchure de l'Hérault site (Fig. 3). Maximum and minimum  $\delta^{13}\text{C}$  values were measured in *C. gigas* (for the sites of Bourgneuf-Couplasse and Les Palles, respectively), while the maximum  $\delta^{15}\text{N}$  value was found for *C. gigas* from Les Palles, and the minimum for *M. galloprovincialis* from Ajaccio-Pointe Parata. Isotopic values are in good agreement with values from the literature (Riera and Richard, 1996a,b; Riera et al., 2002a,b; Lorrain et al., 2002b; Riera, 2007; Lefebvre et al., 2009; Dubois et al., 2014; etc.). Interestingly, *C. gigas* exhibited the largest variability in  $\delta^{13}\text{C}$  (range of 9.5‰ compared to 5.4‰ for *M. edulis* and 4.5‰ for *M. galloprovincialis*). This illustrates the large trophic plasticity of this species, as already reported (Dubois et al., 2007b).

#### 3.2.1. Species versus regional variability

Overall, the data structure (intimately linked to the ROCCH strategy) prevented to clearly separate the species and the geographical (or environmental) effects on this variability, although the results and the statistical tests performed partly allowed highlighting the prevalence of some or the other effect at both large and small spatial scales. Significant differences were found between *M. galloprovincialis* and each of the two other species for C/N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and between *C. gigas* and *M. edulis* for the C/N ratio (all  $p$ -values  $< 0.03$ ; Table 3). Similarly, significant differences were found between the Mediterranean bivalves and the bivalves sampled at each of the two other regions for the three parameters, and between the Atlantic and English Channel bivalves for the C/N ratio (all  $p$ -values  $\leq 0.01$ ; Table 3). It is difficult to attribute the above differences to regional and/or species differences, since regions and species vary simultaneously in our sample collection. Thus, differences were also tested between species within each region and between regions for each species. The Mediterranean region was not tested since *M. galloprovincialis* is the only species represented. Also, *M. galloprovincialis* was not included in the test of inter-specific differences within the English Channel and the Atlantic coast, since this species is found at only two sites outside of the Mediterranean region (Table 1). The only significant differences were found for the C/N ratio between *M. edulis* and *C. gigas* within the Atlantic and within the English Channel region ( $p$ -values  $\leq 0.01$ ; Table 3). Thus, it appears that large scale differences in C/N are rather due to species differences whereas large scale differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are rather due to differences between Mediterranean ecosystems on the one hand and English Channel and Atlantic ecosystems on the other hand. Values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were lower ( $p$ -values  $< 0.001$  and  $p$ -values  $< 0.05$ , respectively) for the Mediterranean bivalves ( $\delta^{15}\text{N} = 5.2 \pm 1.6\text{‰}$ ;  $\delta^{13}\text{C} = -21.3 \pm 1.1\text{‰}$ ) than those for Atlantic and English Channel bivalves ( $\delta^{15}\text{N} = 7.9 \pm 1.1\text{‰}$ ;  $\delta^{13}\text{C} = -20.1 \pm 1.6\text{‰}$ ). This can be related to the oligotrophic regime of the Mediterranean Sea, where diazotrophy occurs. Indeed, dinitrogen ( $\text{N}_2$ ) fixation leads to low  $\delta^{15}\text{N}$  of diazotrophs compared other planktonic autotrophs. Pantoja et al. (2002) measured low  $\delta^{15}\text{N}$  in surface particulate nitrogen ( $2.7 \pm 1.2\text{‰}$  to  $-0.2 \pm 0.7\text{‰}$ ), chlorophyll-a ( $2.6 \pm 2.3\text{‰}$  to  $-7.1 \pm 1.3\text{‰}$ ) and deep-water nitrate ( $3.4 \pm 0.5\text{‰}$  to  $2.5 \pm 0.1\text{‰}$ ) in the eastern Mediterranean Sea, due to the contribution of  $\text{N}_2$  fixation to the water column N budget. An overall North to South decrease in  $\delta^{15}\text{N}$  was also recorded in the coastal POM of the French coast with low and even negative values in the studied sites of the Mediterranean Sea (English Channel/Atlantic ocean  $\delta^{15}\text{N} = 6.2 \pm 1.7\text{‰}$ , Mediterranean sea  $\delta^{15}\text{N} = 3.2 \pm 1.5\text{‰}$ ; Liénart et al., 2017). These low values were explained by the contribution of diazotrophs to the Mediterranean POM (ca. 15% yearly average), whereas no contribution of diazotrophs was recorded in the POM of the Atlantic Ocean and the English Channel (Liénart et al., 2017). Lower  $\delta^{13}\text{C}$  values of Mediterranean bivalves are also consistent with lower  $\delta^{13}\text{C}$  values of Mediterranean POM compared to Atlantic and English Channel values (Liénart et al., 2017).

#### 3.2.2. Smaller scale spatial variability

In order to test if the trophic resources of bivalves were related to their type of ecosystem (categorized as "estuary", "lagoon", "bay",

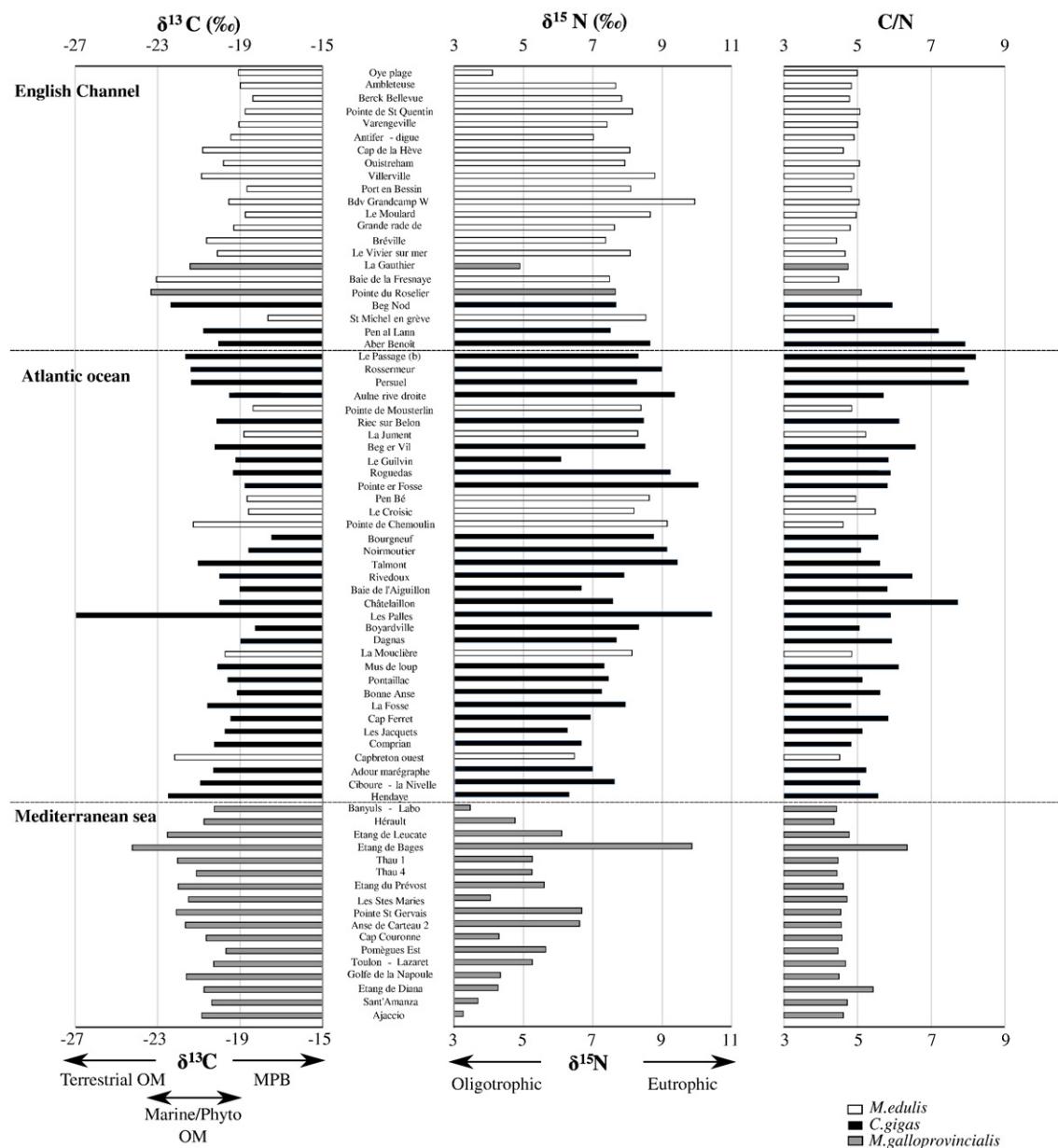


Fig. 3.  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and C/N ratio values for the sampling sites. Arrows represent a global tendency for the evolution of parameters.

Table 3

Results of the pairwise Wilcoxon tests. Med = Mediterranean Sea; En. Chan. = English Channel; *M. gallo* = *M. galloprovincialis*.

$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		C/N			
		<i>M. edulis</i>	<i>C. gigas</i>	<i>M. edulis</i>	<i>C. gigas</i>	<i>M. edulis</i>	<i>C. gigas</i>
All sites	<i>C. gigas</i>	–		<i>C. gigas</i>	–	<i>C. gigas</i>	***
	<i>M. gallo</i>	***	**	<i>M. gallo</i>	***	<i>M. gallo</i>	*
Atlantic	<i>C. gigas</i>			<i>C. gigas</i>			***
	<i>M. edulis</i>	–		<i>M. edulis</i>	–	<i>M. edulis</i>	***
En. Chan.	<i>C. gigas</i>			<i>C. gigas</i>			***
	<i>M. edulis</i>	–		<i>M. edulis</i>	–	<i>M. edulis</i>	**
All species	Atlantic		En. Chan.	Atlantic		Atlantic	En. Chan.
	En. Chan.	–		En. Chan.	–	En. Chan.	Med
<i>M. edulis</i>	En. Chan.	–		En. Chan.	–	En. Chan.	–
	Med	–		Med	***	Med	***
<i>C. gigas</i>	Atlantic			Atlantic		Atlantic	En. Chan.
	En. Chan.	–		En. Chan.	–	En. Chan.	–

– not significant.

\*  $0.01 < \text{p-value} < 0.05$ .

\*\*  $0.001 < \text{p-value} < 0.01$ .

\*\*\*  $\text{p-value} < 0.001$ .

"littoral system"; Table 1), a PERMANOVA was performed for each species. No relationship between bivalve stable isotopes and ecosystem "type" was detected at the study's space scale (PERMANOVA;  $p > 0.05$ ; Fig. 4). Thus, very local environmental conditions also drive bivalve diet. At the local scales ( $\sim 10^1$  km), some sites showed peculiar values indicative of local specificities. Four of such sites are described below and presented in the insects of the Fig. 1. At the site Les Palles, located in the Marennes-Oléron Bay in a channel bordering the Charente estuary, *C. gigas* exhibited the lowest  $\delta^{13}\text{C}$  value ( $-27.0\text{\textperthousand}$ ) and the highest  $\delta^{15}\text{N}$  value (10.4%) of the whole data set (*C. gigas* means for this study:  $-20.2\text{\textperthousand}$  for  $\delta^{13}\text{C}$  and  $8.0\text{\textperthousand}$  for  $\delta^{15}\text{N}$ ). These values are very different from those of the nearby sites within the Marenne-Oléron Bay (Boyardville, Dagnas, Mus de Loup; Fig. 3). This suggests a greater use

of continental/estuarine trophic resources by oysters at the site Les Palles, which is directly under the influence of the Charente River.

Interestingly, the site La Moulière is also located under the direct influence of the Charente River, and is even closer to the mouth of the Charente Estuary than the site Les Palles. However, *M. edulis* that was sampled at this site exhibited a  $\delta^{13}\text{C}$  value of  $-19.7\text{\textperthousand}$  (Fig. 3). Clearly, the two species did not use similar trophic resources even if the two sites are in the same bay and only 3 km apart. The *C. gigas* at the La Fosse site in the lower Gironde estuary exhibited a  $\delta^{13}\text{C}$  value of  $-20.6\text{\textperthousand}$  (Fig. 3), indicating that it mainly used marine material as a trophic resource (marine phytoplankton  $\delta^{13}\text{C} = -20.9\text{\textperthousand}$  in the vicinity of the Gironde estuary, a macrotidal hyperturbid estuary; Savoye et al., 2012). Since POM  $\delta^{13}\text{C}$  ranges between  $-26\text{\textperthousand}$  and  $-23\text{\textperthousand}$  in the lower part of the Gironde estuary (Savoye et al., 2012), the above indicates 1) that, at the site of La Fosse, *C. gigas* feed preferentially at high tide, probably because of the lower turbidity at high compared to low tide, or 2) that *C. gigas* is able to select and/or assimilate selectively phytoplankton within the POM pool. The comparison of the three estuarine sites Les Palles, La Moulière and La Fosse illustrates again the higher trophic plasticity of *C. gigas* compared to *M. edulis*. A third singular site is the Etang de Bages site in the Mediterranean Sea. Bivalves from this coastal lagoon presented a particularly low  $\delta^{13}\text{C}$  value ( $-24.2\text{\textperthousand}$ ) and a high  $\delta^{15}\text{N}$  value (9.9%). This lagoon has already been described as subjected to an organic C and total N enrichment, at least in a specific part of the lagoon where the bivalves are collected, related to anthropogenic inputs (Vouve et al., 2014).

Finally, the turbid macrotidal coastal ecosystem of the Bourgneuf-Coupeplasse site had the highest  $\delta^{13}\text{C}$  value among all *C. gigas* samples ( $-17.5\text{\textperthousand}$ ). In this environment characterized by wide mudflats and a limited macrophytic and phytoplanktonic production, microphytobenthos species are among the most abundant marine food sources of the ecosystem (Meleder et al., 2007). Microphytobenthos may represent up to 50% of the microalgae present in the water column after its resuspension by turbulence and shear stresses generated by tidal currents or wind waves (De Jonge and Van Beusekom, 1995). It may thus contribute significantly to the diet of deposit and suspension feeders in this area, because microphytobenthos usually exhibits the highest  $\delta^{13}\text{C}$  values among potential food sources in temperate coastal ecosystems (i.e., relative to marine phytoplankton or terrestrial material debris for instance; (Riera and Richard, 1996a; Barille et al., 1997; Pinckney et al., 2003).

Overall, we have shown that the environmental settings of the sampling sites are diverse and that these very local specificities have a significant impact on the elemental and isotopic composition in C and N of coastal bivalves. Thus, to understand wide scale C&N elemental and isotopic compositions, detailed knowledge of each sampling site is a prerequisite.

### 3.3. Temporal variability, part II (decadal trends)

Coastal ecosystems offer multiple food sources to filter-feeders. They can be of riverine, estuarine and marine origin, and composed of phytoplankton, microphytobenthos, macrophytes and/or diverse detritus (Dubois et al., 2014). Marine phytoplankton largely contributes to suspension feeders' diet (Dubois et al., 2007b). Changes in the on-shore/off-shore balance of environmental features (water currents and composition, sediment dynamics, coverage of vegetation, etc....) can modify the proportions of respective potential food sources in the environment of primary consumers (Vizzini and Mazzola, 2006; Liénart et al., 2017). Since regional climate that changes at decadal scale (Goberville et al., 2010) impacts this balance, we investigated pluri-decadal variation of bivalve  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and C/N ratio at ten selected sites along the French coastline to quantify these changes. Overall, the evolution of elemental and isotopic signatures over the past three decades showed no generalised pattern but progressive trends and/or rapid shifts between quiescent time spans for some parameters at some sites (Fig. 5).

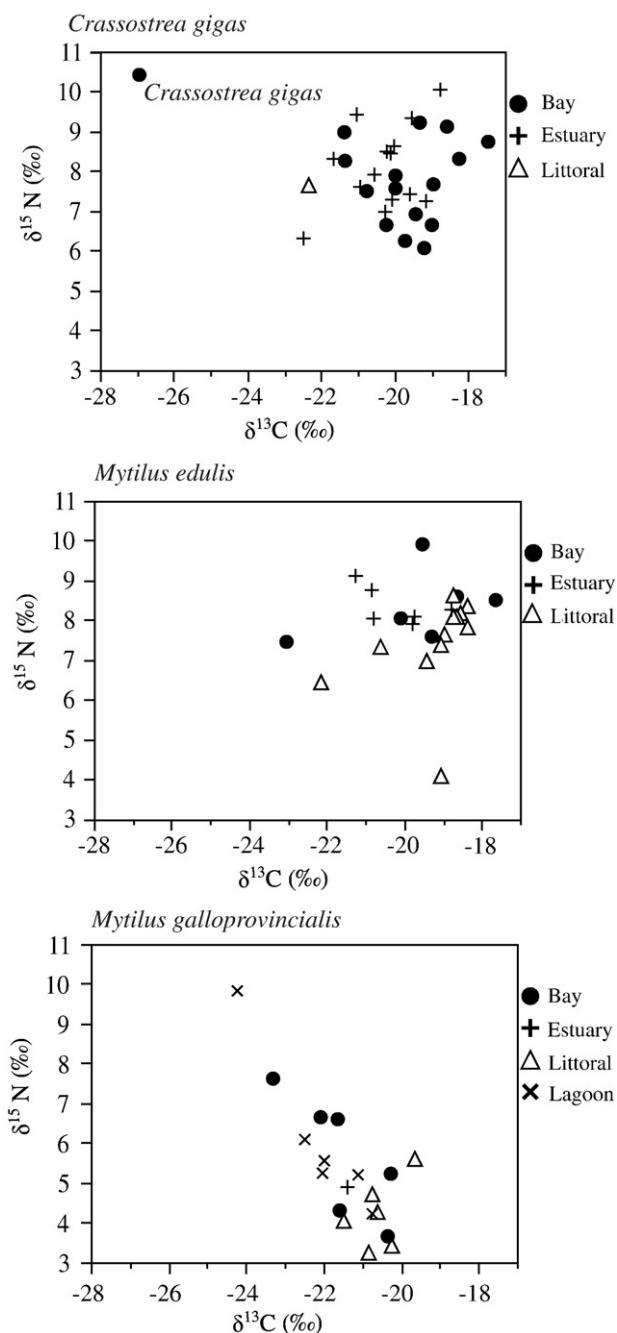


Fig. 4.  $\delta^{15}\text{N}$  vs.  $\delta^{13}\text{C}$  biplots depending on ecosystem types for the three species.

### 3.3.1. C and N elemental and isotopic shifts

The evolution of elemental and isotopic signatures over the past three decades showed no generalised pattern but progressive trends and/or rapid shifts between quiescent time spans for some parameters at some sites (Fig. 5). Considering the three parameters, a total of eleven series out of 30 series (10 sites with 3 parameters) exhibited significant shifts (3 for *M. edulis*, 2 for *C. gigas* and 6 for *M. galloprovincialis*; 4 in  $\delta^{13}\text{C}$ , 4 in  $\delta^{15}\text{N}$  and 3 in C/N). All shifts were negative, with one exception. The values ranged between  $-1.0$  and  $-1.5\text{‰}$  for  $\delta^{13}\text{C}$ ,  $-1.1$  and  $-1.9\text{‰}$  for  $\delta^{15}\text{N}$  and between  $-0.2$  and  $+0.5$  for C/N ratio. Changes were also investigated for river flow, whenever sites were under the influence of rivers, and for environmental parameters (temperature, salinity, chlorophyll a) when available in the vicinity of the study sites. No significant shift was detected for the river flows. Regarding the other available environmental parameters, only one shift was detected: for *M. edulis* near Pointe de Chemoulin, a negative shift in salinity occurred in 1998, concomitantly to a drop in C/N ratio. The drop in observed salinities could suggest an increase in continental POM. This weakly biodegradable terrestrial organic matter could be a potential explanation of the C/N decrease. Nevertheless, the lack of correlation between hydrological parameters near the sites and core parameters cannot lead to the conclusion that there is no link between environmental parameters and bivalve parameters. It rather illustrates the difference in the length of the available data sets and thus the lack of long-term data sets for these environmental parameters near the studied sites.

Most of the shifts in bivalve parameters occurred between 1996 and 2005 (8 out of 11 shifts). Shifts in the early 2000's were also reported for environmental variables in these three regions of the French coastal ocean (Goberville et al., 2010). This decadal evolution was correlated with the evolution of local climate and regional climate indices. At

local scale (Gironde estuary; Chaalali et al., 2013) concomitant shifts occurred at the same period for environmental parameters, local and regional climate, and even fish communities. Correlations between regional climate, environmental parameters and phytoplankton (the main food source for bivalves) communities, with difference in functioning between periods before and after early 2000's, were also reported in English Channel and Atlantic French systems (David et al., 2012; Hernandez-Farinis et al., 2014). In Arcachon Bay, one of the studied system, it has been shown that such shift in phytoplankton community has led to reproduction anomalies for *C. gigas*.

### 3.3.2. Trends in C and N elemental and isotopic ratios

Significant long-term trends were also found for nine of the series (3 for *M. edulis*, 1 for *C. gigas* and 5 for *M. galloprovincialis*; 3 in  $\delta^{13}\text{C}$ , 3 in  $\delta^{15}\text{N}$  and 3 in C/N; Fig. 5). It is not always clear if long-term evolution is due to shift, trend or both since both shifts and trends were detected in eight of the series. All trends were negative, with one exception. Considering that the trends would be linear, slopes would range between  $-0.06$  and  $-0.07\text{‰ yr}^{-1}$  for  $\delta^{13}\text{C}$ ,  $-0.05$  and  $-0.10\text{‰ yr}^{-1}$  for  $\delta^{15}\text{N}$  and between  $-0.03$  and  $+0.03\text{‰ yr}^{-1}$  for C/N ratio. This would lead to change in  $\delta^{13}\text{C}$  by  $-1.6$  to  $-1.9\text{‰}$ , in  $\delta^{15}\text{N}$  by  $-1.4$  to  $-2.7\text{‰}$  and in C/N ratio by  $-0.3$  to  $+0.8$  over the 27 years of the series. Since no time series of environmental parameters was available in the vicinity of the sampling sites for the study period (1987–2014), investigating trends in environmental parameters was not possible. Nevertheless, it has been shown that the temperature of the western Mediterranean Sea surface water has increased following a slope of  $0.03\text{ °C yr}^{-1}$  during the last 3 decades (e.g. Nykjaer, 2009). Also, the Gironde estuary underwent multi-decadal changes with an increase in temperature and salinity, and a decrease in turbidity (David et al., 2007).

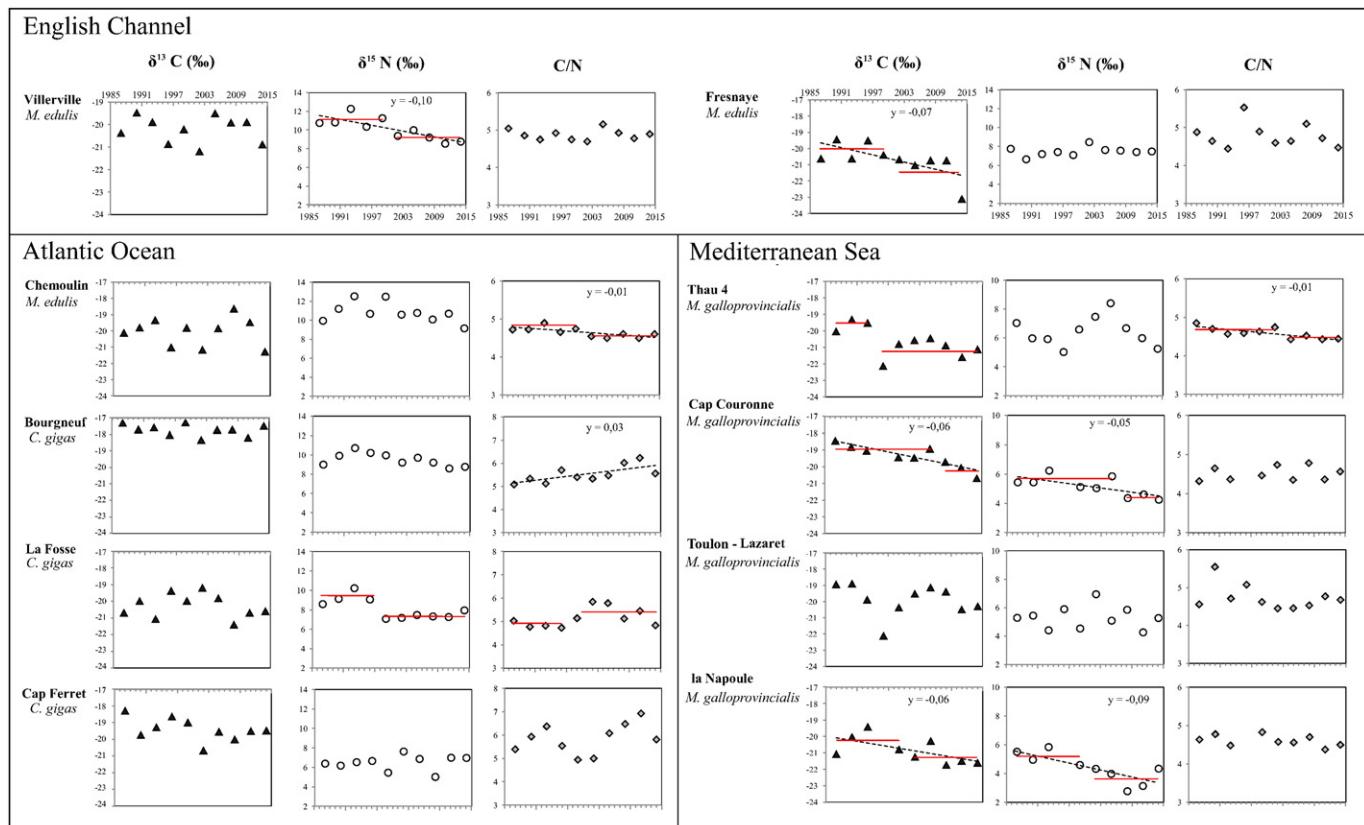


Fig. 5. Values measured between 1987 and 2014 for 10 sites. Red lines = significant shift. Dotted lines = significant trend with (y) for the slope.

### 3.3.3. Bivalve isotopic ratios in the context of global change

Whenever significant ( $p < 0.05$ ), the shift and trends detected in bivalve elemental and isotopic ratios indicated little changes in C/N ratio but quite large decrease in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Changes in bivalve  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  can be due to changes in their own diet and/or changes in that of their trophic resources. For instance, a decrease in bivalve  $\delta^{13}\text{C}$  may indicate a decrease in the proportion of marine sources in bivalve diet. Also, considering to first order that phytoplankton is the main trophic resource for bivalves (e.g. Lefebvre et al., 2009) changes in bivalve  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  may be due to changes in phytoplankton  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Since there is a strong correlation between phytoplankton  $\delta^{13}\text{C}$  and temperature (Fontugne and Duplessy, 1981), the increase in seawater temperature would lead to the increase in phytoplankton  $\delta^{13}\text{C}$ . This cannot explain the decrease in bivalve  $\delta^{13}\text{C}$ . In contrast, the release of anthropogenic  $\text{CO}_2$  in the atmosphere leads to its accumulation in the ocean. Since  $\delta^{13}\text{C}$  of anthropogenic  $\text{CO}_2$  is very low, this results in the decrease of aquatic  $\text{CO}_2$   $\delta^{13}\text{C}$  (Suess effect; (Bacastow et al., 1996) at a rate of  $-0.0282\text{‰ yr}^{-1}$  (Quay et al., 1992)). In turn, this induces a decrease in phytoplankton  $\delta^{13}\text{C}$  at a similar rate and may partly explain the long-term decrease in bivalve  $\delta^{13}\text{C}$  (rate:  $-0.06\text{‰}$  to  $-0.07\text{‰ yr}^{-1}$ ). As mentioned above, climate change induces modifications in phytoplankton diversity, especially an increase in small phytoplankton with temperature increases (David et al., 2012). Small phytoplankton exhibits lower  $\delta^{13}\text{C}$  than large phytoplankton (Popp et al., 1998). This may additionally explain the long-term decrease in bivalve  $\delta^{13}\text{C}$ .

The shifts observed for some of the series in the early 2000's are therefore concomitant with many other shifts observed in French coastal systems that are linked to global climate (see above). Usually, the link between regional climate and biological/ecological response is indirect and follows a cascade of effects such as: regional climate affects local climate and meteorology that affect the hydrological environment that affects (directly or still indirectly) the biological or ecological response (e.g. David et al., 2012; Chaalali et al., 2013; David et al. submitted). Such a cascade effect may have led to the modification of bivalve trophic resources and body condition and/or modification of food source  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , as revealed by long-term evolution in C and N elemental and/or isotopic values of bivalves. However, all series do not exhibit long-term evolution. This clearly indicates that regional-climate effects, if any, are strongly modulated by local drivers.

## 4. Conclusion

In this study, we described for the first time the spatial and temporal variations in C and N elemental and isotopic compositions of bivalves in three regions along the French coasts. We have shown the necessity to take into account the season of sampling to compare data sets. For our study, the C/N ratio of *Mytilus* spp. varies seasonally due to its reproduction cycle, bivalve  $\delta^{13}\text{C}$  varies seasonally, depending of diet seasonality. At large spatial scales, systematic differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were observed between oligotrophic and meso-eutrophic systems due to differences in diet with the likely use of diazotrophs in oligotrophic systems. At local scale, bivalve isotopic ratios did not allow characterising specificities associated to particular ecosystem types (estuary, lagoon, bay, littoral ecosystem). This illustrates the capacity of bivalves to select and/or assimilate selectively food resources of different origins, and exemplified with the large trophic plasticity of *C. gigas*. The investigation of the temporal evolution of C and N elemental and isotopic ratios over three decades revealed shifts and trends for part of the series with decreases in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the early 2000's. At the same time, shifts in regional and local ecosystems were observed for hydrology, phytoplankton and fish communities at local and/or regional scale in the English Channel and Atlantic Ocean coastal ecosystems. The rather weak linkage shows that the cascade of processes between regional and local climate and hydrological environment and bivalve isotopic ratios is modulated and even inhibited by local drivers. This has been observed at the regional ( $10^3\text{ km}$ ) and decadal scale, caution

must then be exerted in ascribing to global change an evolution in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and C/N.

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